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Hybridization in East African *Ranunculus*

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Summary : Field and laboratory studies of *R. aberdaricus*, *R. oreophytus*, and *R. multifidus* on Mt. Kenya included morphological, cytological, and ecological data from 10 populations. From these data and from an examination of additional herbarium materials, it is concluded that *R. aberdaricus* is a stabilized octoploid hybrid species resulting from hybridization between *R. multifidus* and *R. oreophytus*. Ulbrich, in the original descriptions of several taxa from East Africa, described a putative hybrid between *R. multifidus* and *R. oreophytus*, *R.* × *fratum*. Results presented here indicate that the type of this taxon is a plant of *R. oreophytus* var. *stolonifer*.

Résumé : Des études morphologiques, cytologiques et écologiques sur 10 populations de *R. aberdaricus*, *R. oreophytus* et *R. multifidus* du Mt. Kénya ont été faites sur le terrain et au laboratoire. L'auteur conclut que *R. aberdaricus* est un hybride octoploïde stable provenant de l'hybridation entre *R. multifidus* et *R. oreophytus*. Parmi plusieurs taxons d'Afrique orientale, Ulbrich décrivit *R.* × *fratum*, hybride supposé entre *R. multifidus* et *R. oreophytus*. Les résultats présentés ici montrent que le type de ce taxon appartient à *R. oreophytus* var. *stolonifer*.

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In *Ranunculus*, hybridization has been a significant factor in speciation, e.g., in the *R. lappaceus* complex (Briggs, 1962), in alpine *Ranunculus* of New Zealand (Fisher, 1965) and in the *R. montanus* group in Europe (Landolt, 1954). Numerous species throughout the genus are polyploids and hybridization has probably been a factor in the origin of many. In some groups such as the *R. hispidus* complex (Duncan, 1980), however, the presence of octoploidy in some populations of species that are otherwise tetraploid coupled with no morphological differentiation in the cytomorphs indicates that autopolyploidy may have also occurred in this genus.

Hybridization has also been suspected in East African *Ranunculus*. Ulbrich (1930) described *Ranunculus* × *fratum*, *R. aberdaricus*, and *R. oreophytus* var. *stolonifer* with *Ranunculus* × *fratum* treated as a sterile hybrid between *R. oreophytus* Delile and *R. multifidus* Forsk. (= *R. pubescens* Thbg.). These names were based on a series of collections made in East Africa along the Northern Naro Moru River on the western slopes of Mt. Kenya and in the Aberdare Mountains, by Robert & Thomas Fries in 1922. Subsequent floristic works have only briefly mentioned these taxa. In the Flora of Tropical East Africa (Turrill & Milne-Redhead, 1952), *R.* × *fratum* is included with only the infor-

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mation provided by Ulbrich (1930) in the original description. *Ranunculus aberdaricus* was included with only the original collections of Fries & Fries cited. There is a more extended discussion of variation in *R. oreophytus* including mention of var. *stolonifer* but the authors felt they did not understand the basis for the named variants in this species and deferred recognition until further study was made. Hedberg (1957), in a discussion of Afroalpine *Ranunculus*, noted that *R.* × *fratum* may be a hybrid, but that the question could not be answered without field studies supplemented by cytological investigations. Also, he speculated that *R. oreophytus* var. *stolonifer* may have originated by introgression.

During 1978, field work was undertaken on the western slopes of Mount Kenya in order to rediscover populations that corresponded to the collections of Fries & Fries and the descriptions of Ulbrich for *R.* X *fratum*, *R. oreophytus* var. *stolonifer*, and *R. aberdaricus*. In addition, populations of *R. oreophytus* var. *oreophytus* and *R. multifidus* were sought.

HABITATS AND DISTRIBUTION

Ranunculus oreophytus var. *oreophytus* is widespread in East Africa occurring in mountainous areas from northern and central Ethiopia, to the Belgian Congo. *Ranunculus oreophytus* var. *stolonifer* occurs in the Elgon Range and on Mount Kenya. *Ranunculus multifidus* is widespread in tropical Africa, ranging from the Saudi Arabian peninsula to South Africa. *Ranunculus aberdaricus* is know only from the Aberdare Range and Mount Kenya. *Ranunculus* × *fratum* is known only from Mount Kenya.

On the western slopes of Mount Kenya, four vegetation zones occur; the bamboo, ericaceous, *Hagenia-Hypericum*, and alpine (Hedberg, 1951, 1957, 1964; Coe, 1967). On the western slopes, the Naro Moru track (Fig. 1) traverses the bamboo zone to the lower edge of the *Hagenia-Hypericum* zone, at elevations from approximately 7,000 ft. to 10,100 ft. Dominants in the bamboo zone are *Arundinaria alpina* and *Podocarpus milanjianus*. Above the bamboo zone, a narrow zone between 10,000 and 11,000 ft. is dominated by *Hagenia abyssinica* and several species of *Hypericum*. Tree line occurs at the upper edge of this zone. Above this zone, *Erica* and *Phillipia* dominate to approximately 12,500 ft. followed by an alpine zone dominated by *Senecio keniodendron*, *S. brassica*, *Lobelia keniensis*, and *L. telekii* in the Teleki Valley, with the upper limit of vegetation occurring at ca. 16,000 ft.

Populations of *Ranunculus multifidus* and *R. aberdaricus* were observed in the bamboo zone. Additional herbarium specimens examined indicate that *R. multifidus* occurs exclusively in this zone although *R. aberdaricus* has been noted at elevations of between 11,000 and 12,000 ft. in other valleys or Mount Kenya and in the Aberdare Mountains.

Ranunculus oreophytus var. *oreophytus* occurs in the alpine zone and is one of the most common herbs in the Teleki Valley from approximately 12,000 to 14,000 ft. In these areas it occurs along stream courses and in dense wet soils on the valley floor. It is also common in other valleys. One population occurring around the edge of a glacial tarn at the head of Hausberg Valley was studied (population 10, Fig. 1, Table 1). *Ranunculus oreophytus* var. *stolonifer* occurs into the *Hagenia-Hypericum* zone and the bamboo

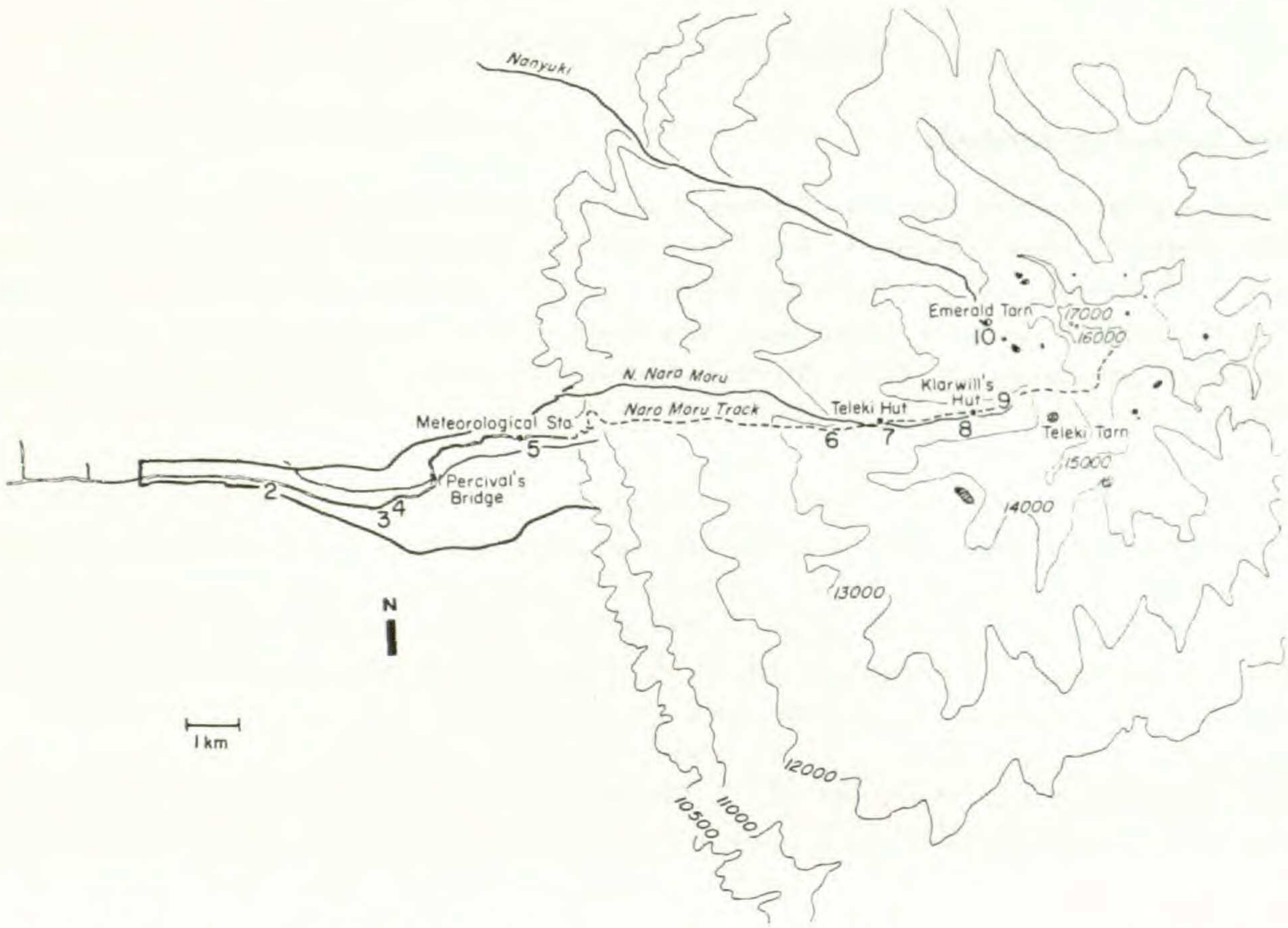

FIG. 1. — Western slopes of Mount Kenya showing collecting localities as numbered. Area above 10,500 ft. contour line within Mount Kenya National Park. Heavy dark line below this elevation outlines the lower boundary of the park. Locations for each population given in Table 1.

zone. Along the western slopes of Mount Kenya, individuals of *R. oreophytus* var. *stolonifer* are distinctly stoloniferous whereas *R. oreophytus* var. *oreophytus* in the alpine zone is acaulescent. Along the Naro Moru Track between approximately 1.5 miles above the National Park gate and Percival's Bridge (Fig. 1) populations of all three species were present. In this area of overlap in the bamboo zone, *R. oreophytus* var. *stolonifer*, *R. multifidus* and *R. aberdaricus* were noted in disturbed vegetation along roadsides and in clearings.

Initial field observations indicated that populations corresponding to the descriptions and types of *R. aberdaricus* appeared intermediate in many morphological characters compared to *R. oreophytus* and *R. multifidus*. The populations were suspected to be of hybrid origin. Populations with individuals corresponding to the type and descriptions of *R.* × *fratum* appeared to represent a stoloniferous form of *R. oreophytus* and corresponded also to previous collections named *R. oreophytus* var. *stolonifer*. The purpose of this paper is to report laboratory studies of collections made on Mount Kenya and herbarium studies of additional collections to investigate further these hypotheses.

MATERIALS AND METHODS

Data gathering methods

Eleven samples from ten populations were gathered along the Naro Moru Track and along the Teleki Valley (Table 1, Fig. 1). All but population 1 are located within the boundaries of Mount Kenya National Park, on the Equator at ca. 37°15′ E longitude. From each population, mass collections of inflorescences, infructescences, and vegetative material were preserved in FAA for morphological study. Herbarium specimens were prepared and vouchers have been deposited at EA, K, and UC. Flower buds were preserved in Newcomer's solution and meiotic chromosomes were studied using the standard acetocarmine squash method. Pollen viability was estimated by using percent stained in lactophenol cotton blue. Notes on the habitats and associated plants for each population were recorded. For each of the 11 samples, 10 vegetative, floral, and fruit characters (Table 2) were measured in each of five individuals. Observations were recorded for each individual on the angle of curvature of the fruiting pedicel, the number and development of the petals, the presence of stolons, and the shape and arrangement of leaflets.

Specimens from BM, EA, G, K, P, and UPS of *R. multifidus*, *R. oreophytus* var. *oreophytus*, *R. oreophytus* var. *stolonifer*, *R.* × *fratum*, and *R. aberdaricus* were examined. The holotypes of the taxa described by Ulbrich were destroyed in Berlin (B) in 1943. However, photos and duplicate collections from the above institutions have been examined.

Data analytic techniques

To analyse the 10 quantitative characters measured on each of five individuals from 11 population samples, principal components analysis was used. Before analysis the values for these characters were standardized using the condensation formula given in Sneath & Sokal (1973). Principal components analysis is described in Harris (1975). The use of this multivariate technique for studies of the relationship and identification of putative hybrid populations has been discussed by Neff & Smith (1979) and Anderson & Harrison (1979). Plots of the first two components were compared to the scoring of qualitative characters to investigate the morphological intermediacy of individuals of putative hybrid origin.

Based on stainability of pollen with cotton blue, the percentage of viable and non-viable pollen were calculated. To test for significant differences in percentage of non-viable pollen, these percentages were compared using a t-test with arcsin transformation of the percentage of non-viable pollen. This technique is discussed in Sokal & Rohlf (1969 : 607-608). A single percentage was calculated for each of the three taxa and all three pairs of taxa were compared.

TABLE 1 : Population samples used in principal component analysis.

SITE NUMBER	TAXON	LOCALITY, COLLECTION NUMBER, AND MEIOTIC CHROMOSOME NUMBER
1	*multifidus*	Along Naro Moru Track ca. 1 km below Mt. Kenya National Park Ranger Station ; elev. 7,900 ft. *Duncan 2795.* $n = 16$.
2	*aberdaricus*	Along Naro Moru Track ca. 5 km below Meterological Station ; elev. 8,700 ft. *Duncan 2794.* $n = 32$.
3	*multifidus*	Along Naro Moru Track ca. 1 km below Percival's Bridge ; elev. 9,000 ft. *Duncan 2792.* $n = 16$.
4A	*aberdaricus*	Along Naro Moru Track ca. 1 km below Percival's Bridge ; elev. 9,000 ft. *Duncan 2789* site 2. $n = 32$.
4B	*aberdaricus*	Along Naro Moru Track ca. 0.8 km below Percival's Bridge ; elev. 9,000 ft. *Duncan 2789* site 1. $n = 32$.
5	*oreophytus* var. *stolonifer*	Along Naro Moru Track ca. 0.1 km above Meterological Station ; elev. 10,000 ft. *Duncan 2764.* $n = 16$.
6	*oreophytus* var. *oreophytus*	At base of Teleki Valley ca. 1 km below Teleki Hut ; elev. 13,000 ft. *Duncan 2780.* $n = 16$.
7	*oreophytus* var. *oreophytus*	At base of Teleki Valley at Teleki Hut ; elev. 13,500 ft. *Duncan 2771.* $n = 16$.
8	*oreophytus* var. *oreophytus*	Along Teleki Valley at Klarwill's Hut ; elev. 13,690 ft. *Duncan 2779.* $n = 16$.
9	*oreophytus* var. *oreophytus*	At head of Teleki Valley at American Camp near Teleki Tarn ; elev. 14,000 ft. *Duncan 2778.* $n = 16$.
10	*oreophytus* var. *oreophytus*	At head of Hausberg Valley around margin of Emerald Tarn ; elev. 14,500 ft. *Duncan 2773.* $n = 16$.

RESULTS

Principal components analysis

The variance in the first two components accounts for approximately 71 percent of the variance in the original data matrix. The plot of these two components is shown in Figure 2. This plot shows the relationships between the 55 individuals on the basis of the values each possesses for the characters most highly correlated with these components (Table 2).

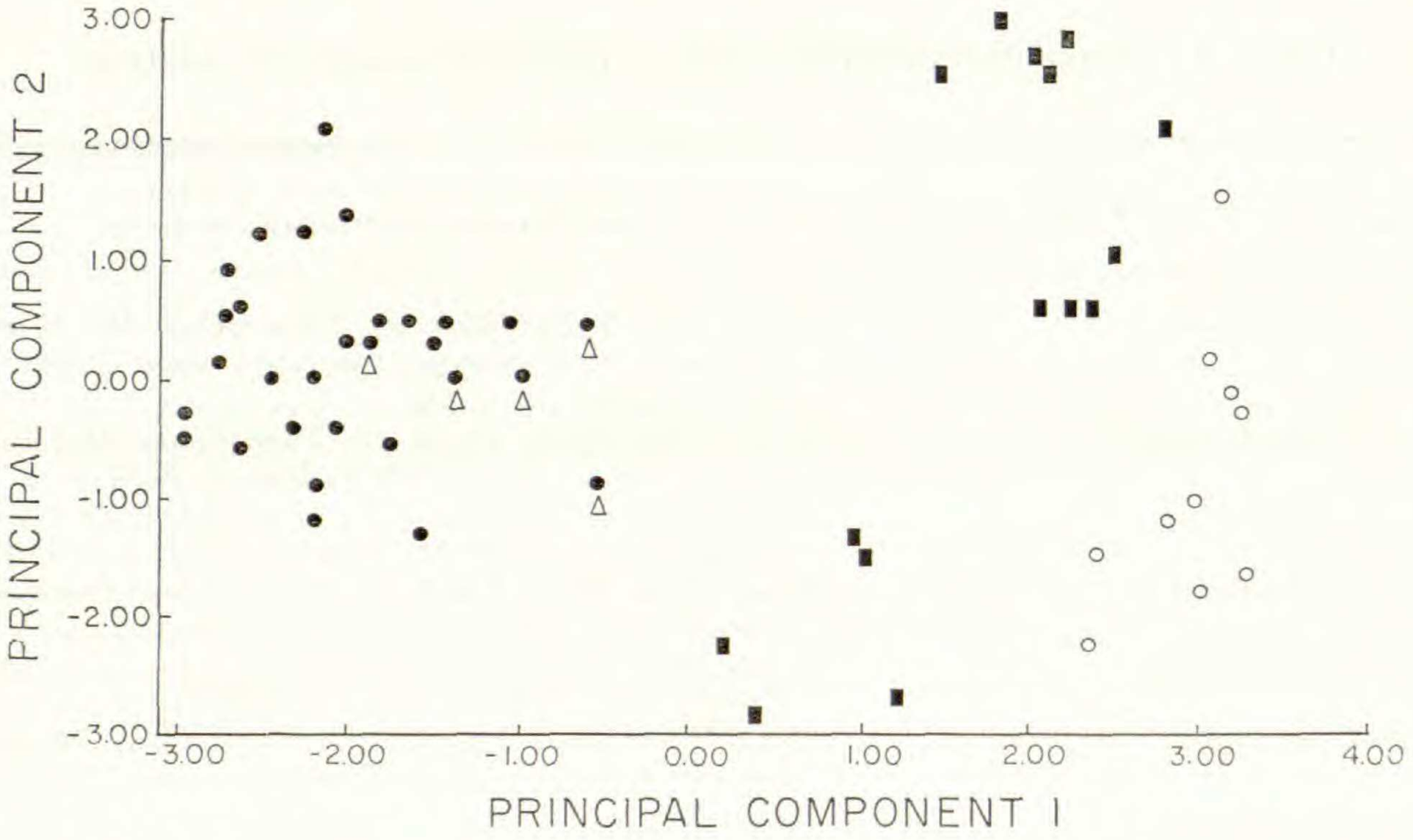

FIG. 2. — Principal components graph for 55 individuals of **Ranunculus multifidus** (open circles), **R aberdaricus** (squares), **R. oreophytus** var. **oreophytus** (closed circles), and **R. oreophytus** var. **stolonifer** (triangles).

TABLE 2 : Morphological character used in principal components analysis and the correlation of each to components 1 and 2.

CHARACTER	PC 1	PC 2
1. Flowering shoot length	0.42	–0.03
2. Peduncle/pedicel length	0.84	0.03
3. Number of teeth/leaflet	0.85	–0.15
4. Sepal length	–0.80	0.21
5. Petal length	–0.92	0.18
6. Petal width at widest point	–0.58	0.62
7. Achene length	0.53	0.76
8. Achene width at widest point	0.73	0.54
9. Achene beak length	–0.41	0.73
10. Achene margin width	0.78	0.37

Principal component one accounts for approximately 50 percent of the variance in the original data set. *Ranunculus oreophytus* possesses the shortest peduncles, number of teeth per leaflet, the narrowest achenes with the narrowest margins, the longest and widest petals and the longest sepals whereas *R. multifidus* possesses the other extreme for these characters and *R. aberdaricus* intermediate along this component (Table 2). Prin-

cipal component two, accounting for approximately 21 percent of the variance in the data set, is correlated most highly with achene length and achene beak length (Table 2). The individuals of each species vary more or less continuously along this axis. Individuals of *R. oreophytus* var. *stolonifer* are placed with individuals of *R. oreophytus* var. *oreophytus* along both components.

Qualitative characters

All individuals of *R. oreophytus* are geocarpic. The peduncle reflexes at maturity and the infructescence is placed in the soil. The peduncles of all individuals of *R. multifidus* are erect at the time of fruiting. Individuals of *R. aberdaricus* display an intermediate condition ; the peduncle arches to approximately 90° to 120° of vertical and the infructescence does not come into contact with the soil. These conditions are shown diagrammatically in Figure 3.

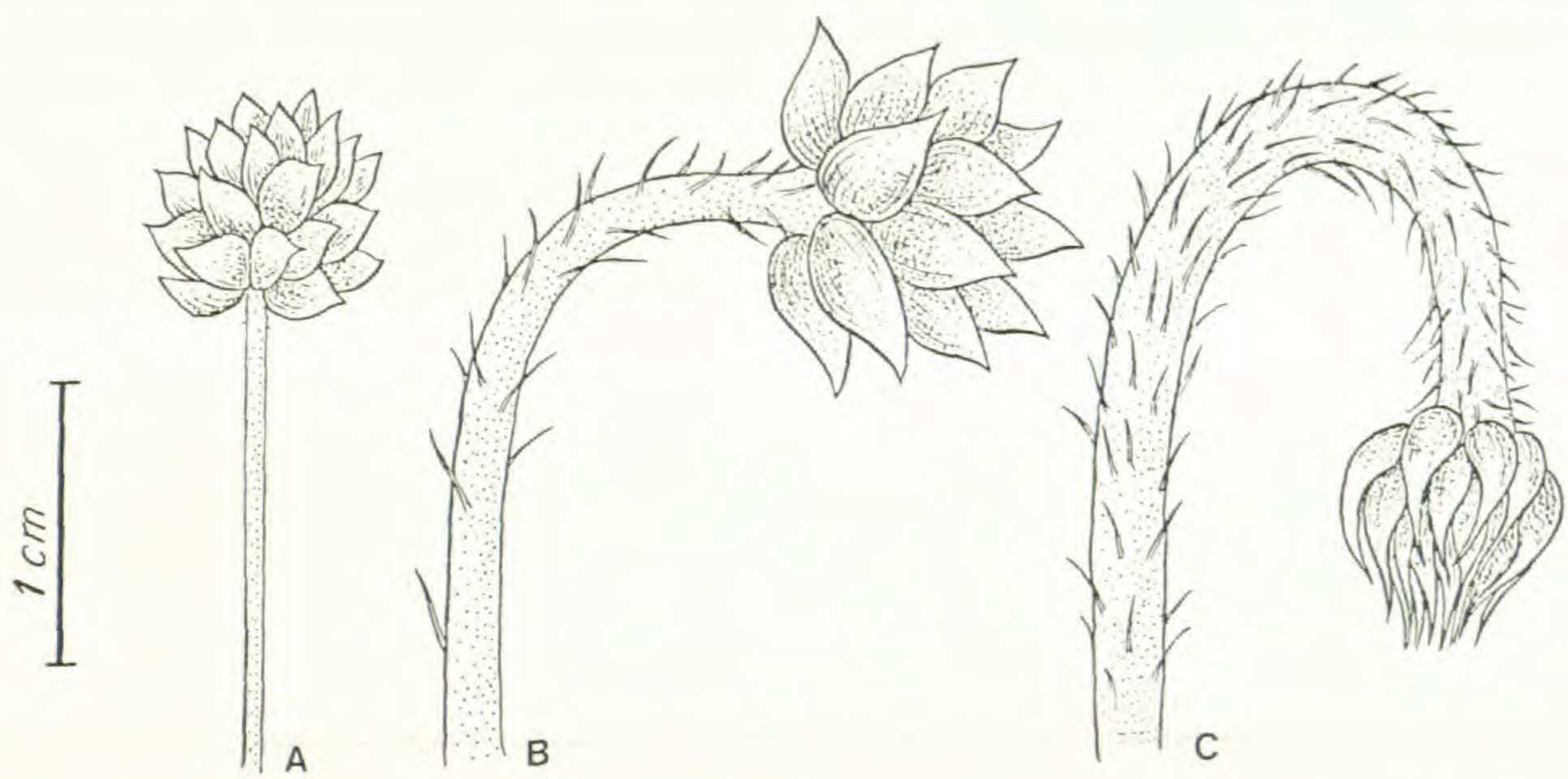

Fig. 3. — Diagrammatic representation of curvature of infructescences of **Ranunculus multifidus** (A), **R. aberdaricus** (B) and **R. oreophytus** (C).

Petal development is frequently abnormal in *R. aberdaricus*. The majority of individuals have five petals per flower. In many flowers only three petals are formed and are frequently misshaped. In all individuals of *R. multifidus* and *R. oreophytus* no abnormal petal development was noted. Stolons were noted in *R. oreophytus* var. *stolonifer* and in *R. aberdaricus* but not in *R. multifidus* or *R. oreophytus* var. *oreophytus*.

The leaves of individuals of both varieties of *R. oreophytus* are pinnately compound with cuneate leaflets. The leaves of *R. multifidus* are once to twice ternately compound with oval to ovate leaflets. Individuals of *R. aberdaricus* have ternately compound leaves with ovate leaflets.

Herbarium studies

Type specimens and photographs for *R. oreophytus* var. *stolonifer*, *R. aberdaricus*, and *R.* × *fratum* were compared to the population samples collected on Mount Kenya. The type of *R.* × *fratum* corresponds to individuals of *R. oreophytus* in petal length and width. Also, the type of *R.* × *fratum* has pinnately compound leaves. A comparison of the isotype of *R.* × *fratum* and a photograph of the holotype of *R. oreophytus* var. *stolonifer* indicate that these names should be considered synonyms. The type of *R. aberdaricus* matches the individuals that are intermediate in several of their morphological features compared to *R. multifidus* and *R. oreophytus*.

Cytological studies

Mount Kenya populations of both *R. oreophytus* var. *oreophytus*, *R. oreophytus* var. *stolonifer*, and *R. multifidus* are tetraploids with 16 pairs of chromosomes at meiosis (Table 1). Populations of *R. aberdaricus* are octoploid with 32 pairs of chromosomes at meiosis. Dividing anther cells also were octoploid with 64 chromosomes. Meiosis appears normal in all populations.

Table 3 : Pollen viability for each species and t-statistic for each pair of species.

Species	Stained (viable)	Unstained (non-viable)	Σ	P (unstained)
1. *oreophytus*	2653	571	3224	0.177
2. *aberdaricus*	367	217	584	0.372
3. *multifidus*	1518	294	1812	0.162

$t_{1,3} = 1.61$ not significant
$t_{2,3} = 7.432$ $p < 0.001$
$t_{1,2} = 7.632$ $p < 0.001$

The percent viable pollen noted in populations of each species is given in Table 3. *Ranunculus aberdaricus* is the most variable over the samples taken but has a fairly high percent viable pollen as measured by stainability in cotton blue. However, the average is approximately 63 %, 20 % lower than in *R. oreophytus* and *R. multifidus*. The t statistic for the comparisons of each pair of taxa shows that *R. aberdaricus* has a significantly higher percentage of non-viable pollen than both *R. multifidus* and *R. oreophytus* ($t = 7.43$ and 7.63, respectively, Table 3). *R. multifidus* and *R. oreophytus* are not statistically different in terms of percent non-viable pollen produced (Table 3).

DISCUSSION

Although Ulbrich (1930) was correct when he suspected hybridization between *R. multifidus* and *R. oreophytus*, the plant he named as *R.* × *fratum* and considered to be a hybrid between these species, is instead a rather large individual of *R. oreophytus* var. *stolonifer*. Ulbrich based his conclusion concerning hybridization on the supposed intermediacy in leaf characters of *R.* × *fratum* compared to *R. multifidus* and *R. oreophytus*. The isotype (K) clearly has pinnately compound leaves. This specimen does not have any of the features of *R. multifidus* with its once to twice three-foliolate leaf. In addition, Ulbrich noted the large size of the individual collected by Fries & Fries. However, a great amount of variation exists in aerial shoot length in *R. oreophytus*. Normally acaulescent individuals of these species from the alpine zone will form well-developed non-stoloniferous aerial shoots when transplanted to lower elevations (Coe, 1967). Finally he indicated that the flowers suggest those of *R. oreophytus*. Measurement on the isotype indicates that petal length and shape are as found in all populations of *R. oreophytus* examined.

The cytological data results do not support the contention of Hedberg that *R. oreophytus* var. *stolonifer* resulted from introgression. A tetraploid chromosome number with normal pairing indicates that the plants are not introgressants.

From intermediacy on the principal components plot and intermediacy of peduncle arching, it is inferred that *R. aberdaricus* has probably evolved by hybridization between *R. oreophytus* and *R. multifidus*. In addition, the presence of an octoploid meiotic chromosome complement compared to the tetraploid level of the putative parents, statistically significantly reduced pollen viability, and developmental abnormalities in the petals further suggest establishment of fertility following chromosome doubling in an originally sterile hybrid. However, allopolyploidy has not resulted in normal development and a level of fertility comparable to the parents although meiosis appears normal.

Habitat preferences and altitudinal/elevational ranges of each species also support the contention that *R. aberdaricus* arose by hybridization. On Mount Kenya, *R. oreophytus* and *R. multifidus* overlap in altitudinal distribution and *R. aberdaricus* occurring primarily in the zone of overlap although previous collections have been made to altitude/elevation of approximately 12,000 ft. Where these species occur together, *Ranunculus aberdaricus* occupies the most disturbed areas and its abundance has probably been increased by improvements to the Naro Moru Track and continued disturbance along this road. Although *R. multifidus* and *R. oreophytus* also occur along the Naro Moru Track, populations of these species occur in areas of denser less frequently disturbed vegetation. The occurrence of specimens of *R. aberdaricus* at higher elevations may be the result of migration and establishment in these areas by individuals preadapted to higher elevations by the combination of genomes from *R. multifidus* and *R. oreophytus*.

The combined occurrence of these species on Mount Kenya does not support a conclusion that the initial hybridization occurred there. Because the same combination of species occurs in the Aberdare Mountains, 50 miles to the west, it is possible that independent formations of this hybrid derivative could have occurred or that *R. aberdaricus* arose in the Aberdares and the current situation on Mount Kenya is the result of later dispersal.

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